526 Rec'd PCT/PTO 06 JUL 2000

FORM PTO-1390 (REV 11-98)	U S DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE	ATTORNEY'S DOCKET NUMBER 3525-86
TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371		U S APPLICATION NO (If known, see 37 C F R 1.5) 09/582863 Unassigned
INTERNATIONAL APPLICATION NO. PCT/SE00/00756	INTERNATIONAL FILING DATE 19 April 2000	PRIORITY DATE CLAIMED 21 April 1999 and 3 December, 1999

TITLE OF INVENTION

NEW USE

APPLICANT(S) FOR DO/EO/US

GUSTAFSSON, David

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.

2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.

3. ☒ This is an express request to **DELAY** national examination procedures (35 U.S.C. 371(f) until the expiration of the applicable time limit set in 35 U.S.C. 371(b) Articles 22 and 39(1).

4. ☐ A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.

5. ☐ A copy of the International Application as filed (35 U.S.C. 371(c)(2)).

   a. ☒ is transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☐ has been transmitted by the International Bureau.
   c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).

6. ☐ A translation of the International Application into English (35 U.S.C. 371(c)(2)).

7. ☐ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3)).

   a. ☐ are transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☐ have been transmitted by the International Bureau.
   c. ☐ have not been made; however, the time limit for making such amendments has **NOT** expired.
   d. ☐ have not been made and will not be made.

8. ☐ A translation of the amendments to the claims under PCT Article 19 (U.S.C. 371(c)(3)).

9. ☒ An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).

10. ☐ A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11. To 16. Below concern document(s) or information included:

11. ☐ An Information Disclosure Statement under 37 C.F.R. 1.97 and 1.98.

12. ☒ An assignment document for recording. A separate cover sheet in compliance with 37 C.F.R. 3.28 and 3.31 is included.

13. ☒ A FIRST preliminary amendment.
    ☐ A SECOND or SUBSEQUENT preliminary amendment.

14. ☐ A substitute specification.

15. ☐ A change of power of attorney and/or address letter.

16. ☐ Other items or information.

U.S. APPLICATION NO. (If known, see 37 C.F.R. 1.5)	INTERNATIONAL APPLICATION NO	ATTORNEY'S DOCKET NUMBER
Unassigned 09/582863	PCT/SE00/00756	3525-86

17. ☒ The following fees are submitted:

	CALCULATIONS PTO USE ONLY
BASIC NATIONAL FEE (37 C.F.R. 1.492(a)(1)-(5):	
-- Neither international preliminary examination fee (37 C.F.R. 1.482) nor international search fee (37 C.F.R. 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO $970.00	
-- International preliminary examination fee (37 C.F.R. 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO $840.00	
-- International preliminary examination fee (37 C.F.R. 1.482) not paid to USPTO but international search fee (37 C.F.R. 1.445(a)(2) paid to USPTO $690.00	
-- International preliminary examination fee paid to USPTO (37 C.F.R. 1.482) but all claims did not satisfy provisions of PCT Article 33(1)-(4) $670.00	
-- International preliminary examination fee paid to USPTO (37 C.F.R. 1.482) and all claims satisfied provisions of PCT Article 33(1)-(4) $96.00	

ENTER APPROPRIATE BASIC FEE AMOUNT =						$	970.00
Surcharge of $130.00 for furnishing the oath or declaration later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 C.F.R. 1.492(e)).						$	0.00
CLAIMS	NUMBER FILED		NUMBER EXTRA	RATE			
Total Claims	19	-20 =	0	X	$18.00	$	0.00
Independent Claims	4	-3 =	1	X	$78.00		78.00
MULTIPLE DEPENDENT CLAIMS(S) (if applicable)					$260.00	$	0.00
TOTAL OF ABOVE CALCULATIONS =						$	**1048.00**
Reduction by ½ for filing by small entity, if applicable. A Small Entity Statement must also be filed (Note 37 C.F.R. 1.9, 1.27, 1.28).							0.00
SUBTOTAL =						$	**1048.00**
Processing fee of $130.00, for furnishing the English Translation later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 C.F.R. 1.492(f)). +							0.00
TOTAL NATIONAL FEE =						$	**1048.00**
Fee for recording the enclosed assignment (37 C.F.R. 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 C.F.R. 3.28, 3.31). **$40.00** per property +						$	40.00
Fee for Petition to Revive Unintentionally Abandoned Application ($1210.00 – Small Entity = $605.00)						$	0.00
TOTAL FEES ENCLOSED =						$	**1088.00**
						Amount to be: refunded	$
						Charged	$

a. ☒ A check in the amount of $1088.00 to cover the above fees is enclosed.
b. ☐ Please charge my Deposit Account No. 14-1140 in the amount of $_____ to cover the above fees. A duplicate copy of this form is enclosed.
c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 14-1140. A duplicate copy of this form is enclosed.
d. ☐ The entire content of the foreign application(s), referred to in this application is/are hereby incorporated by reference in this application.

NOTE: Where an appropriate time limit under 37 C.F.R. 1.494 or 1.495 has not been met, a petition to revive (37 C.F.R. 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

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SIGNATURE

Leonard C. Mitchard
NAME

29,009 July 6, 2000
REGISTRATION NUMBER Date

440425

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of

GUSTAFSSON, David

Serial No. Unassigned

Filed: July 6, 2000

For: NEW USE

Atty. Ref.: 3525-86

Group:

Examiner:

* * * * * * * * * * *

July 6, 2000

Assistant Commissioner for Patents
Washington, DC 20231

PRELIMINARY AMENDMENT

Sir:

Please amend the above application as follows:

IN THE CLAIMS

Claim 3, line 1, delete "or Claim 2".

Claim 5, line 1, delete "any one of Claims 1 to 4" and replace by --Claim 1--.

Claim 8, line 1, delete "any one of the preceding claims" and replace by --Claim 1--.

Claim 9, lines 1-2, delete "any one of Claims 1 to 8" and replace by --Claim 1--.

Claim 10, line 1, delete "any one of Claims 1 to 8" and replace by --Claim 1--.

Claim 15, line 1, delete "any one of Claims 12 to 14" and replace by --Claim 12--.

Claim 18, line 1, delete "or Claim 17".

REMARKS

The above amendments have been made to place the application in a more traditional format.

Respectfully submitted,

NIXON & VANDERHYE P.C.

By: _____
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Title: **NEW USE**

Reference: H 2094-1

Inventors: David Gustafsson

NEW USE

Field of the Invention

This invention relates to a new use of low molecular weight thrombin inhibitors.

Background and Prior Art

Blood coagulation is the key process involved in both haemostasis (i.e. the prevention of blood loss from a damaged vessel) and thrombosis (i.e. the formation of a blood clot in a blood vessel, sometimes leading to vessel obstruction).

Coagulation is the result of a complex series of enzymatic reactions. One of the ultimate steps in this series of reactions is the conversion of the proenzyme prothrombin to the active enzyme thrombin.

Thrombin is known to play a central role in coagulation. It activates platelets, leading to platelet aggregation, converts fibrinogen into fibrin monomers, which polymerise spontaneously into fibrin polymers, and activates factor XIII, which in turn crosslinks the polymers to form insoluble fibrin. Furthermore, thrombin activates factor V and factor VIII leading to a "positive feedback" generation of thrombin from prothrombin.

Effective inhibitors of thrombin are thus known, and/or are expected, to be useful as anticoagulants and therefore useful in the therapeutic treatment of thrombosis and related disorders.

The early development of low molecular weight inhibitors of thrombin has been described by Claesson in Blood Coagul. Fibrinol. (1994) **5**, 411. Low molecular weight thrombin inhibitors have been described more recently in US Patent N° 4,346,078; International Patent Applications WO 93/11152, WO 93/18060, WO 93/05069, WO 94/20467, WO 94/29336, WO 95/35309, WO 95/23609, WO 96/03374, WO 96/06832, WO 96/06849, WO 96/25426, WO 96/32110, WO 97/01338, WO 97/02284, WO 97/15190, WO 97/30708, WO 97/40024, WO 97/46577, WO 98/06740, WO 97/49404, WO 97/11693, WO 97/24135, WO 97/47299, WO 98/01422, WO 98/57932, WO 99/29664, WO 98/06741, WO 99/37668, WO 99/37611, WO 98/37075, WO 99/00371, WO 99/28297, WO 99/29670, WO 99/40072, WO 99/54313, WO 96/31504, WO 00/01704 and WO 00/08014; and European Patent Applications 648 780, 468 231, 559 046, 641 779, 185 390, 526 877, 542 525, 195 212, 362 002, 364 344, 530 167, 293 881, 686 642, 669 317, 601 459 and 623 596.

In particular, international patent application WO 94/29336 discloses a group of compounds, including HOOC-CH_2-(*R*)Cgl-Aze-Pab-H (in which Cgl represents cyclohexylglycine, Aze represents *S*-azetidine-2-carboxylic acid and Pab-H represents 4-aminomethyl-amidinobenzene), which is also known as melagatran (see Example 1 of WO 94/29336). International Patent Application WO 97/23499 discloses prodrugs of *inter alia* melagatran.

None of the above-mentioned documents disclose or suggest the administration of an active thrombin inhibitor in conjunction with a prodrug of that thrombin inhibitor, or indeed in conjunction with a prodrug of any thrombin inhibitor.

Deep venous thrombosis (DVT) and pulmonary embolism (PE) are major health problems, which may give rise to serious outcomes. In particular, PE may be fatal, or may result in the development of pulmonary hypertension and heart failure from recurrent embolism. DVT may result in post-thrombotic venous insufficiency and ulcers in the affected part of the body (e.g. leg). Both are common conditions, which have a great impact on worldwide healthcare costs.

There is a considerable incidence of DVT and PE following orthopaedic surgery. For example, in patients undergoing total hip replacement, the incidence of DVT in the absence of thromboprophylaxis may be as high as 45 to 57%. Further, the incidence of proximal DVT may be between 23 and 36%, and that of fatal PE, 0.34 to 6%. In patients undergoing total knee replacement in the absence of thromboprophylaxis, the postoperative incidence of DVT is between 40 and 84%, of proximal DVT is between 9 and 20%, and of fatal PE is between 0.2 and 0.7%. In patients undergoing general surgery in the absence of thromboprophylaxis, the postoperative incidence of DVT is about 25%. (Reference: Chest (1998) **114**, 531S to 560S.)

Low-dose, subcutaneous (s.c.) unfractionated heparin is the most widely used current prophylactic treatment for venous thromboembolism resulting from orthopaedic and general surgery. The incidence of DVT after total

hip replacement has been shown to be reduced (see Chest reference above).

The use of low-molecular weight heparin (LMWH) in the prophylaxis of DVT following total hip and knee replacement operations has been shown to further the reduce incidence (when compared to low dose unfractionated heparin), without a concomitant increase in bleeding (see Chest reference above).

However, prolonged treatment with heparins has been shown to give rise to an increased risk of osteoporosis. Heparins may also give rise to "heparin-induced thrombocytopenia" (HIT), are dependent on the plasma level of the endogenous thrombin inhibitor, antithrombin, and do not inactivate clot-bound thrombin.

Oral anticoagulants, such as warfarin (a vitamin K antagonist), has also been shown to be effective in reducing DVT after major surgery (see Chest reference above). However, due to the risk of bleeding, and the need for frequent laboratory control, the use of this substance is generally reserved for high risk patients, and/or for long term use. Vitamin K antagonists also demonstrate a notable risk of interaction with other drugs and certain foods, and their use requires monitoring of the patient's blood coagulation status.

Antiplatelet agents, such as aspirin, have been shown to have limited efficacy in preventing DVT (see Chest reference above).

Comparative clinical studies carried out during the course of total hip replacement operations have shown that subcutaneous administration of

the thrombin inhibitor hirudin is superior to unfractionated heparin and LMWH in reducing the frequency of total and proximal DVT with no corresponding increase in bleeding (see Eriksson *et al* in Lancet, **347**, 635 (1996) and J. Bone Joint. Surg., Sep., 11 (1996)). However, hirudin is expensive and has an immunogenic potential.

Thus, there is a need for effective treatments of thrombotic conditions such as DVT.

Disclosure of the Invention

We have found, surprisingly, that administration of a low molecular weight thrombin inhibitor in conjunction with a prodrug of a (or a prodrug of that) thrombin inhibitor gives rise to a notable anticoagulant effect.

According to a first aspect of the invention there is provided a kit of parts comprising components:

(a) a pharmaceutical formulation including a low molecular weight thrombin inhibitor, or a pharmaceutically acceptable derivative thereof, in admixture with a pharmaceutically acceptable adjuvant, diluent or carrier; and

(b) a pharmaceutical formulation including a prodrug of a low molecular weight thrombin inhibitor, or a pharmaceutically acceptable derivative of that prodrug, in admixture with a pharmaceutically acceptable adjuvant, diluent or carrier,

which components (a) and (b) are each provided in a form that is suitable for administration in conjunction with the other.

It is preferred that the prodrug of component (b) is a prodrug of the active low molecular weight thrombin inhibitor of component (a).

According to a further aspect of the invention, there is provided a method of making a kit of parts as defined herein, which method comprises bringing a component (a), as defined above, into association with a component (b), as defined above, thus rendering the two components suitable for administration in conjunction with each other.

By bringing the two components "into association with" each other, we include that components (a) and (b) may be:

(i) provided as separate formulations (i.e. independently of one another), which are subsequently brought together for use in conjunction with each other in combination therapy; or

(ii) packaged and presented together as separate components of a "combination pack" for use in conjunction with each other in combination therapy.

Thus, there is further provided a kit of parts comprising:

(1) one of components (a) and (b) as defined herein; together with

(2) instructions to use that component in conjunction with the other of the two components.

The kits of parts defined herein may comprise more than one formulation including an appropriate quantity/dose of thrombin inhibitor, and/or more than one formulation including an appropriate quantity/dose of respective prodrug, in order to provide for repeat dosing. If more than one formulation (comprising thrombin inhibitor or prodrug) is present, such

formulations may be the same, or may be different in terms of the dose of thrombin inhibitor/prodrug, chemical composition and/or physical form.

A further aspect of the invention provides a method of treatment of a condition in which inhibition of thrombin is required or desired, which comprises administration of:

(a) a pharmaceutical formulation including a low molecular weight thrombin inhibitor, or a pharmaceutically acceptable derivative thereof, in admixture with a pharmaceutically acceptable adjuvant, diluent or carrier; in conjunction with

(b) a pharmaceutical formulation including a prodrug of a low molecular weight thrombin inhibitor, or a pharmaceutically acceptable derivative of that prodrug, in admixture with a pharmaceutically acceptable adjuvant, diluent or carrier,

to a patient suffering from, or susceptible to, such a condition.

For the avoidance of doubt, as used herein, the term "treatment" includes therapeutic and/or prophylactic treatment.

"Pharmaceutically acceptable derivatives" of thrombin inhibitors and prodrugs includes salts (e.g. pharmaceutically acceptable non-toxic organic or inorganic acid addition salts) and solvates. It will be appreciated that the term pharmaceutically acceptable derivatives of active thrombin inhibitors includes those derivatives that have the same biological function and/or activity as that thrombin inhibitor but, for the purposes of this invention, does not include prodrugs of that thrombin inhibitor.

By "administration in conjunction with", we include that respective formulations comprising thrombin inhibitor and/or prodrug are administered, sequentially, separately and/or simultaneously, over the course of treatment of the relevant condition, which condition may be acute or chronic. Preferably, the term includes that the two formulations are administered (optionally repeatedly) sufficiently closely in time for there to be a beneficial effect for the patient, that is greater, over the course of the treatment of the relevant condition, than if either of the two formulations are administered (optionally repeatedly) alone, in the absence of the other formulation, over the same course of treatment. Determination of whether a combination provides a greater beneficial effect in respect of, and over the course of treatment of, a particular condition, will depend upon the condition to be treated or prevented, but may be achieved routinely by the skilled person.

Thus, the term "in conjunction with" includes that one or other of the two formulations may be administered (optionally repeatedly) prior to, after, and/or at the same time as, administration with the other component. When used in this context, the terms "administered simultaneously" and "administered at the same time as" include that individual doses of thrombin inhibitor and prodrug are administered within 48 hours (e.g. 24 hours) of each other.

Components (a) and (b) as described herein may also be presented (i.e. formulated) as a combined preparation (i.e. presented as a single formulation including low molecular thrombin inhibitor and prodrug).

Thus, there is further provided a pharmaceutical formulation including a low molecular weight thrombin inhibitor (or a pharmaceutically acceptable

derivative thereof) and a prodrug of a low molecular weight thrombin inhibitor (or a pharmaceutically acceptable derivative of that prodrug), in admixture with a pharmaceutically acceptable adjuvant, diluent or carrier.

The term "low molecular weight thrombin inhibitor" will be understood by those skilled in the art. The term may also be understood to include any composition of matter (e.g. chemical compound) which inhibits thrombin to an experimentally determinable degree in *in vivo* and/or in *in vitro* tests, and which possesses a molecular weight of below 2,000, preferably below 1,000.

Preferred low molecular weight thrombin inhibitors include low molecular weight peptide-based, amino acid-based, and/or peptide analogue-based, thrombin inhibitors.

The term "low molecular weight peptide-based, amino acid-based, and/or peptide analogue-based, thrombin inhibitors" will be well understood by one skilled in the art to include low molecular weight thrombin inhibitors with one to four peptide linkages, and includes those described in the review paper by Claesson in Blood Coagul. Fibrin. (1994) **5**, 411, as well as those disclosed in US Patent N° 4,346,078; International Patent Applications WO 93/11152, WO 93/18060, WO 93/05069, WO 94/20467, WO 94/29336, WO 95/35309, WO 95/23609, WO 96/03374, WO 96/06832, WO 96/06849, WO 96/25426, WO 96/32110, WO 97/01338, WO 97/02284, WO 97/15190, WO 97/30708, WO 97/40024, WO 97/46577, WO 98/06740, WO 97/49404, WO 97/11693, WO 97/24135, WO 97/47299, WO 98/01422, WO 98/57932, WO 99/29664, WO 98/06741, WO 99/37668, WO 99/37611, WO 98/37075, WO 99/00371, WO 99/28297, WO 99/29670, WO 99/40072, WO 99/54313, WO 96/31504, WO

00/01704 and WO 00/08014; and European Patent Applications 648 780, 468 231, 559 046, 641 779, 185 390, 526 877, 542 525, 195 212, 362 002, 364 344, 530 167, 293 881, 686 642, 669 317, 601 459 and 623 596, the disclosures in all of which documents are hereby incorporated by reference.

Preferred low molecular weight peptide-based thrombin inhibitors include HOOC-CH_2-(*R*)Cha-Pic-Nag-H (wherein Cha represents cyclohexylalanine, Pic represents (*S*)-pipecolinic acid and Nag represents noragmatine; known as inogatran; see International Patent Application WO 93/11152) and, especially, HOOC-CH_2-(*R*)Cgl-Aze-Pab-H (known as melagatran; see above and International Patent Application WO 94/29336).

The term "prodrug" of a low molecular weight thrombin inhibitor includes any compound that, following oral or parenteral administration, is metabolised *in vivo* to form a low molecular weight thrombin inhibitor (as defined herein), in an experimentally-detectable amount, and within a predetermined time (e.g. within a dosing interval of between 6 and 24 hours (i.e. once to four times daily)), following oral or parenteral administration. Prodrugs of the thrombin inhibitor melagatran that may be mentioned include those disclosed in international patent application WO 97/23499. Preferred prodrugs are those of the formula R^1O_2C-CH_2-(*R*)Cgl-Aze-Pab-OH (see the list of abbreviations above or in WO 97/23499), wherein R^1 represents C_{1-10} alkyl or benzyl, such as linear or branched C_{1-6} alkyl (e.g. C_{1-4} alkyl, especially methyl, propyl and, particularly, ethyl) and the OH group replaces one of the amidino hydrogens in Pab.

The term "condition in which inhibition of thrombin is required or desired" will be understood by those skilled in the art to include the following:

The treatment and/or prophylaxis of thrombosis and hypercoagulability in blood and tissues of animals including man. It is known that hypercoagulability may lead to thrombo-embolic diseases. Conditions associated with hypercoagulability and thrombo-embolic diseases which may be mentioned include inherited or acquired activated protein C resistance, such as the factor V-mutation (factor V Leiden), and inherited or acquired deficiencies in antithrombin III, protein C, protein S, heparin cofactor II. Other conditions known to be associated with hypercoagulability and thrombo-embolic disease include circulating antiphospholipid antibodies (Lupus anticoagulant), homocysteinemi, heparin induced thrombocytopenia and defects in fibrinolysis.

The treatment of conditions where there is an undesirable excess of thrombin without signs of hypercoagulability, for example in neurodegenerative diseases such as Alzheimer's disease.

Particular disease states which may be mentioned include the therapeutic and/or prophylactic treatment of venous thrombosis (e.g. DVT) and pulmonary embolism, arterial thrombosis (e.g. in myocardial infarction, unstable angina, thrombosis-based stroke and peripheral arterial thrombosis) and systemic embolism usually from the atrium during arterial fibrillation or from the left ventricle after transmural myocardial infarction, or caused by congestive heart failure; prophylaxis of re-occlusion (ie thrombosis) after thrombolysis, percutaneous trans-luminal

angioplasty (PTA) and coronary bypass operations; the prevention of re-thrombosis after microsurgery and vascular surgery in general.

Further indications include the therapeutic and/or prophylactic treatment of disseminated intravascular coagulation caused by bacteria, multiple trauma, intoxication or any other mechanism; anticoagulant treatment when blood is in contact with foreign surfaces in the body such as vascular grafts, vascular stents, vascular catheters, mechanical and biological prosthetic valves or any other medical device; and anticoagulant treatment when blood is in contact with medical devices outside the body such as during cardiovascular surgery using a heart-lung machine or in haemodialysis; the therapeutic and/or prophylactic treatment of idiopathic and adult respiratory distress syndrome, pulmonary fibrosis following treatment with radiation or chemotherapy, septic shock, septicemia, inflammatory responses, which include, but are not limited to, edema, acute or chronic atherosclerosis such as coronary arterial disease, cerebral arterial disease, peripheral arterial disease, reperfusion damage, and restenosis after percutaneous trans-luminal angioplasty (PTA).

Preferred conditions include thrombosis, especially DVT, including distal and proximal DVT. The present invention finds particular utility in the prophylactic treatment of DVT resulting from surgery, such as gastrointestinal, or orthopaedic, surgery (e.g. hip or knee replacement). This includes DVT resulting from immobilisation after surgery.

In accordance with the invention, thrombin inhibitors, prodrugs of thrombin inhibitors, and derivatives of either, may be administered orally, intravenously, subcutaneously, buccally, rectally, dermally, nasally, tracheally, bronchially, topically, by any other parenteral route, or *via*

inhalation, in the form of a pharmaceutical preparation comprising the thrombin inhibitor or prodrug in a pharmaceutically acceptable dosage form. Depending on the disorder, and the patient, to be treated, as well as the route of administration, the compositions may be administered at varying doses.

Preferred modes of delivery are systemic. For melagatran and derivatives thereof, preferred modes of administration are parenteral, more preferably intravenous, and especially subcutaneous. For prodrugs of melagatran, preferred modes of administration are oral.

In the therapeutic treatment of mammals, and especially humans, thrombin inhibitors, prodrugs of thrombin inhibitors, and derivatives of either will generally be administered as a pharmaceutical formulation in admixture with a pharmaceutically acceptable adjuvant, diluent or carrier, which may be selected with due regard to the intended route of administration and standard pharmaceutical practice.

Suitable formulations for use in administering thrombin inhibitors are known in the art, and include those known from US Patent N° 4,346,078; International Patent Applications WO 93/11152, WO 93/18060, WO 93/05069, WO 94/20467, WO 94/29336, WO 95/35309, WO 95/23609, WO 96/03374, WO 96/06832, WO 96/06849, WO 96/25426, WO 96/32110, WO 97/01338, WO 97/02284, WO 97/15190, WO 97/30708, WO 97/40024, WO 97/46577, WO 98/06740, WO 97/49404, WO 97/11693, WO 97/24135, WO 97/47299, WO 98/01422, WO 98/57932, WO 99/29664, WO 98/06741, WO 99/37668, WO 99/37611, WO 98/37075, WO 99/00371, WO 99/28297, WO 99/29670, WO 99/40072, WO 99/54313, WO 96/31504, WO 00/01704 and WO 00/08014; and

European Patent Applications 648 780, 468 231, 559 046, 641 779, 185 390, 526 877, 542 525, 195 212, 362 002, 364 344, 530 167, 293 881, 686 642, 669 317, 601 459 and 623 596, the disclosures in all of which documents are hereby incorporated by reference.

Suitable formulations for use with melagatran, derivatives and prodrugs thereof are described in the literature, for example as described in *inter alia* international patent applications WO 94/29336, WO 96/14084, WO 96/16671, WO 97/23499, WO 97/39770, WO 97/45138, WO 98/16252, WO 99/27912 and WO 99/27913, the disclosures in which documents are hereby incorporated by reference. Otherwise, the preparation of suitable formulations may be achieved non-inventively by the skilled person using routine techniques.

The amounts of thrombin inhibitor, prodrug, or derivative of either, in the formulation will depend on the severity of the condition, and on the patient, to be treated, as well as the compound(s) which is/are employed, but may be determined non-inventively by the skilled person.

Suitable doses of thrombin inhibitors, prodrugs and derivatives of either, in the therapeutic and/or prophylactic treatment of mammalian, especially human, patients may be determined routinely by the medical practitioner or other skilled person, and include the respective doses discussed in the prior art documents disclosing thrombin inhibitors that are mentioned hereinbefore, the disclosures in which are hereby incorporated by reference.

In the case of melagatran, suitable doses of active compound, prodrugs and derivatives thereof, in the therapeutic and/or prophylactic treatment of

mammalian, especially human, patients include those which give a mean plasma concentration of up to 5 μmol/L, for example in the range 0.001 to 5 μmol/L over the course of treatment of the relevant condition. Suitable doses may thus be in the range 0.1 mg once daily to 25 mg three times daily, and/or up to 100 mg infused parenterally over a 24 hour period, for melagatran, and in the range 0.1 mg once daily to 100 mg three times daily for prodrugs of melagatran including those specifically mentioned hereinbefore.

In any event, the physician, or the skilled person, will be able to determine the actual dosage which will be most suitable for an individual patient, which is likely to vary with the condition that is to be treated, as well as the age, weight, sex and response of the particular patient to be treated. The above-mentioned dosages are exemplary of the average case; there can, of course, be individual instances where higher or lower dosage ranges are merited, and such are within the scope of this invention.

The sequence in which the formulations comprising thrombin inhibitor, and prodrug, may be administered (i.e. whether, and at what point, sequential, separate and/or simultaneous administration takes place) may be determined by the physician or skilled person. For example, the sequence may depend upon many factors that will be evident to the skilled person, such as whether, at any time during the course or period of treatment, one or other of the formulations cannot be administered to the patient for practical reasons (e.g. the patient is unconscious and thus unable to take an oral formulation comprising either thrombin inhibitor or prodrug).

For example, in the treatment of thrombosis (e.g. DVT) resulting from surgery, such as gastrointestinal, or orthopaedic, surgery, and when the active thrombin inhibitor is melagatran, it is preferred that the formulation comprising melagatran is administered parenterally within two days (e.g. within 24 hours) of surgery (either prior to or after surgery), and particularly immediately prior to (e.g. within 2 hours), and/or within up to 12 hours after, surgery (e.g. at least one hour after surgery), and thereafter for up to between 3 and 7 (e.g. between 0 and 2, such as between 1 and 2) days after that surgery, and that the formulation comprising prodrug is administered orally within 7 days following that surgery (preferably once administration of melagatran has been terminated) for up to e.g. between 11 and 40 days, preferably 9 days, more preferably up to 8 days.

The method described herein may have the advantage that, in the treatment of conditions in which inhibition of thrombin is required or desired, it may be more convenient for the physician and/or patient than, be more efficacious than, be less toxic than, have a broader range of activity than, be more potent than, produce fewer side effects than, or that it may have other useful pharmacological properties over, similar methods known in the prior art for the treatment of such conditions.

The invention is illustrated, but in no way limited, by the following example.

Example 1

Clinical Trial - Melagatran and EtOOC-CH_2-(*R*)Cgl-Aze-Pab-OH Combination Therapy

A controlled, randomised, parallel group, Swedish multi-centre pilot study was carried out. The study was open with regard to the drugs under evaluation but was blind for the patients, all personnel at the study sites, and for the person monitoring the experiments with regard to the doses of melagatran and the prodrug of melagatran, EtOOC-CH_2-(*R*)Cgl-Aze-Pab-OH (P; see WO 97/23499).

Dalteparin (Fragmin®; Pharmacia-Upjohn) was used as a reference compound.

Patients scheduled for primary elective total hip or knee replacement were eligible for inclusion, and were randomly selected into one of three groups, each to receive different doses of melagatran and P, or dalteparin. In all, 135 patients were included in the study, of which 105 patients could be used for evaluation with respect to thromboembolic events using central assessment of locally performed phlebograms.

About 32 patients in each treatment group were evaluated according to the protocol. A stratified randomisation, by centre and type of surgery, was used to ensure that approximately equal numbers of patients were given each of the drugs under evaluation at all participating centres (in all six centres were used) for both types of surgery (hip or knee). Each centre received study drugs in blocks of four, separately for hips and knees. Within each block, the order of the study drugs was randomised.

The following formulations were used in the study:

Melagatran – 5, 10 or 20 mg/mL in aqueous saline solution.

P - appropriate weight (see below) in a tablet also comprising 59 to 63 mg corn starch, 115 mg microcrystalline cellulose and 2 mg sodium stearyl fumarate.

The following doses of melagatran and P were used in the study:

Treatment A - s.c. melagatran (1 mg) b.i.d. for 2 days, followed by oral administration of P (6 mg) b.i.d. for 6 to 9 days.

Treatment B - s.c. melagatran (2 mg) b.i.d. for 2 days, followed by an oral administration of P (12 mg) b.i.d. for 6 to 9 days.

Treatment C - s.c. melagatran (4 mg) b.i.d. for 2 days, followed by an oral administration of P (24 mg) b.i.d. for 6 to 9 days.

The patients receiving melagatran and P received treatment on the day of surgery. The patient received the first injection after induction of anaesthesia immediately before surgery. For knee-patients, the pre-operative melagatran injection was given before tourniquets were applied. The second injection was given in the evening the same day. The patient received one melagatran injection in the morning and one in the evening over the next 24 hours, until oral administration of P, twice daily, started. The first oral dose of P was always taken in the morning. Thus, the total treatment period was between 8 and 11 days.

Treatment D - dalteparin (Fragmin®): one s.c. injection of 5000 U during the evening of the day before surgery, continuing with one s.c. injection every evening over a treatment period of 8 to 11 days.

The plasma concentrations of melagatran were recorded.

The results of the trial, in terms of the frequencies of thromboembolism after hip or knee surgery, are tabulated below:

	Treatment A		Treatment B		Treatment C		Treatment D	
	(n)	(%)	(n)	(%)	(n)	(%)	(n)	(%)
Outcome	6/29	21	6/24	25	4/24	16	5/27	19

These data show that a combination of subcutaneously administered melagatran and orally administered P is effective in preventing DVT after orthopaedic surgery.

Claims

1. A kit of parts comprising:

(a) a pharmaceutical formulation including a low molecular weight thrombin inhibitor, or a pharmaceutically acceptable derivative thereof, in admixture with a pharmaceutically acceptable adjuvant, diluent or carrier; and

(b) a pharmaceutical formulation including a prodrug of a low molecular weight thrombin inhibitor, or a pharmaceutically acceptable derivative of that prodrug, in admixture with a pharmaceutically acceptable adjuvant, diluent or carrier,

which components (a) and (b) are each provided in a form that is suitable for administration in conjunction with the other.

2. A kit of parts as claimed in Claim 1, wherein the prodrug of component (b) is a prodrug of the thrombin inhibitor of component (a).

3. A kit of parts as claimed in Claim 1 or Claim 2, wherein components (a) and (b) are suitable for sequential, separate and/or simultaneous use in the treatment of a condition in which inhibition of thrombin is required or desired.

4. A kit of parts as claimed in Claim 3, wherein the condition is deep venous thrombosis.

5. A kit of parts as claimed in any one of Claims 1 to 4, wherein the thrombin inhibitor is melagatran.

6. A kit of parts as claimed in Claim 5, wherein the prodrug is of the formula

$$R^1O_2C\text{-}CH_2\text{-}(R)Cgl\text{-}Aze\text{-}Pab\text{-}OH,$$

wherein R^1 represents linear or branched C_{1-6} alkyl and the OH group replaces one of the amidino hydrogens in Pab.

7. A kit of parts as claimed in Claim 6, wherein R^1 represents methyl, ethyl or propyl.

8. A kit of parts as claimed in any one of the preceding claims, wherein the formulation comprising thrombin inhibitor, or derivative thereof, is a parenteral formulation and that comprising the prodrug, or derivative thereof, is an oral formulation.

9. A method of making a kit of parts as defined in any one of Claims 1 to 8, which method comprises bringing a component (a) according to any one of Claims 1 to 8, into association with a component (b) according to any one of Claims 1 to 8, thus rendering the two components suitable for administration in conjunction with each other.

10. A kit of parts comprising:
(1) one of components (a) and (b) as defined in any one of Claims 1 to 8; together with
(2) instructions to use that component in conjunction with the other of the two components.

11. A pharmaceutical formulation including a low molecular weight thrombin inhibitor (or a pharmaceutically acceptable derivative thereof) and a prodrug of a low molecular weight thrombin inhibitor (or a

pharmaceutically acceptable derivative of that prodrug), in admixture with a pharmaceutically acceptable adjuvant, diluent or carrier.

12. A method of treatment of a condition in which inhibition of thrombin is required or desired, which comprises administration of:

(a) a pharmaceutical formulation including a low molecular weight thrombin inhibitor, or a pharmaceutically acceptable derivative thereof, in admixture with a pharmaceutically acceptable adjuvant, diluent or carrier; in conjunction with

(b) a pharmaceutical formulation including a prodrug of a low molecular weight thrombin inhibitor, or a pharmaceutically acceptable derivative of that prodrug, in admixture with a pharmaceutically acceptable adjuvant, diluent or carrier,

to a patient suffering from, or susceptible to, such a condition.

13. A method as claimed in Claim 12 in which component (a) is administered prior to commencement of administration of component (b).

14. A method of treatment of a condition in which inhibition of thrombin is required or desired, which comprises administration of a formulation as defined in Claim 11 to a patient suffering from, or susceptible to, such a condition.

15. A method as claimed in any one of Claims 12 to 14, wherein the condition is deep venous thrombosis.

16. A method as claimed in Claim 15, wherein the thrombosis results from surgery.

17. A method as claimed in Claim 16, wherein the surgery is gastrointestinal surgery or orthopaedic surgery.

18. A method as claimed in Claim 16 or Claim 17, wherein component (a) is administered parenterally prior to and/or after surgery and component (b) is administered orally following that surgery.

19. The use of a thrombin inhibitor, or a pharmaceutically acceptable derivative thereof, in the manufacture of a medicament for the treatment or prophylaxis of a condition in which inhibition of thrombin is required or desired, which treatment or prophylaxis comprises administration of:

(a) a pharmaceutical formulation including a low molecular weight thrombin inhibitor, or a pharmaceutically acceptable derivative thereof, in admixture with a pharmaceutically acceptable adjuvant, diluent or carrier; in conjunction with

(b) a pharmaceutical formulation including a prodrug of a low molecular weight thrombin inhibitor, or a pharmaceutically acceptable derivative of that prodrug, in admixture with a pharmaceutically acceptable adjuvant, diluent or carrier,

to a patient suffering from, or susceptible to, such a condition.

A PHARMACEUTICAL FORMULATION COMPRISING A LOW MOLECULAR WEIGHT THROMBIN INHIBITOR AND ITS PRODRUG.

Field of the Invention

This invention relates to a new use of low molecular weight thrombin inhibitors.

Background and Prior Art

Blood coagulation is the key process involved in both haemostasis (i.e. the prevention of blood loss from a damaged vessel) and thrombosis (i.e. the formation of a blood clot in a blood vessel, sometimes leading to vessel obstruction).

Coagulation is the result of a complex series of enzymatic reactions. One of the ultimate steps in this series of reactions is the conversion of the proenzyme prothrombin to the active enzyme thrombin.

Thrombin is known to play a central role in coagulation. It activates platelets, leading to platelet aggregation, converts fibrinogen into fibrin monomers, which polymerise spontaneously into fibrin polymers, and activates factor XIII, which in turn crosslinks the polymers to form insoluble fibrin. Furthermore, thrombin activates factor V and factor VIII leading to a "positive feedback" generation of thrombin from prothrombin.

Effective inhibitors of thrombin are thus known, and/or are expected, to be useful as anticoagulants and therefore useful in the therapeutic treatment of thrombosis and related disorders.

The early development of low molecular weight inhibitors of thrombin has been described by Claesson in Blood Coagul. Fibrinol. (1994) 5, 411. Low molecular weight thrombin inhibitors have been described more recently in US Patent N° 4,346,078; International Patent Applications WO 93/11152, WO 93/18060, WO 93/05069, WO 94/20467, WO 94/29336, WO 95/35309, WO 95/23609, WO 96/03374, WO 96/06832, WO 96/06849, WO 96/25426, WO 96/32110, WO 97/01338, WO 97/02284, WO 97/15190, WO 97/30708, WO 97/40024, WO 97/46577, WO 98/06740, WO 97/49404, WO 97/11693, WO 97/24135, WO 97/47299, WO 98/01422, WO 98/57932, WO 99/29664, WO 98/06741, WO 99/37668, WO 99/37611, WO 98/37075, WO 99/00371, WO 99/28297, WO 99/29670, WO 99/40072, WO 99/54313, WO 96/31504, WO 00/01704 and WO 00/08014; and European Patent Applications 648 780, 468 231, 559 046, 641 779, 185 390, 526 877, 542 525, 195 212, 362 002, 364 344, 530 167, 293 881, 686 642, 669 317, 601 459 and 623 596.

In particular, international patent application WO 94/29336 discloses a group of compounds, including HOOC-CH_2-(*R*)Cgl-Aze-Pab-H (in which Cgl represents cyclohexylglycine, Aze represents *S*-azetidine-2-carboxylic acid and Pab-H represents 4-aminomethyl-amidinobenzene), which is also known as melagatran (see Example 1 of WO 94/29336). International Patent Application WO 97/23499 discloses prodrugs of *inter alia* melagatran.

None of the above-mentioned documents disclose or suggest the administration of an active thrombin inhibitor in conjunction with a prodrug of that thrombin inhibitor, or indeed in conjunction with a prodrug of any thrombin inhibitor.

Deep venous thrombosis (DVT) and pulmonary embolism (PE) are major health problems, which may give rise to serious outcomes. In particular, PE may be fatal, or may result in the development of pulmonary hypertension and heart failure from recurrent embolism. DVT may result in post-thrombotic venous insufficiency and ulcers in the affected part of the body (e.g. leg). Both are common conditions, which have a great impact on worldwide healthcare costs.

There is a considerable incidence of DVT and PE following orthopaedic surgery. For example, in patients undergoing total hip replacement, the incidence of DVT in the absence of thromboprophylaxis may be as high as 45 to 57%. Further, the incidence of proximal DVT may be between 23 and 36%, and that of fatal PE, 0.34 to 6%. In patients undergoing total knee replacement in the absence of thromboprophylaxis, the postoperative incidence of DVT is between 40 and 84%, of proximal DVT is between 9 and 20%, and of fatal PE is between 0.2 and 0.7%. In patients undergoing general surgery in the absence of thromboprophylaxis, the postoperative incidence of DVT is about 25%. (Reference: Chest (1998) **114**, 531S to 560S.)

Low-dose, subcutaneous (s.c.) unfractionated heparin is the most widely used current prophylactic treatment for venous thromboembolism resulting from orthopaedic and general surgery. The incidence of DVT after total

hip replacement has been shown to be reduced (see Chest reference above).

The use of low-molecular weight heparin (LMWH) in the prophylaxis of DVT following total hip and knee replacement operations has been shown to further the reduce incidence (when compared to low dose unfractionated heparin), without a concomitant increase in bleeding (see Chest reference above).

However, prolonged treatment with heparins has been shown to give rise to an increased risk of osteoporosis. Heparins may also give rise to "heparin-induced thrombocytopenia" (HIT), are dependent on the plasma level of the endogenous thrombin inhibitor, antithrombin, and do not inactivate clot-bound thrombin.

Oral anticoagulants, such as warfarin (a vitamin K antagonist), has also been shown to be effective in reducing DVT after major surgery (see Chest reference above). However, due to the risk of bleeding, and the need for frequent laboratory control, the use of this substance is generally reserved for high risk patients, and/or for long term use. Vitamin K antagonists also demonstrate a notable risk of interaction with other drugs and certain foods, and their use requires monitoring of the patient's blood coagulation status.

Antiplatelet agents, such as aspirin, have been shown to have limited efficacy in preventing DVT (see Chest reference above).

Comparative clinical studies carried out during the course of total hip replacement operations have shown that subcutaneous administration of

the thrombin inhibitor hirudin is superior to unfractionated heparin and LMWH in reducing the frequency of total and proximal DVT with no corresponding increase in bleeding (see Eriksson *et al* in Lancet, **347**, 635 (1996) and J. Bone Joint. Surg., Sep., 11 (1996)). However, hirudin is expensive and has an immunogenic potential.

Thus, there is a need for effective treatments of thrombotic conditions such as DVT.

Disclosure of the Invention

We have found, surprisingly, that administration of a low molecular weight thrombin inhibitor in conjunction with a prodrug of a (or a prodrug of that) thrombin inhibitor gives rise to a notable anticoagulant effect.

According to a first aspect of the invention there is provided a kit of parts comprising components:

(a) a pharmaceutical formulation including a low molecular weight thrombin inhibitor, or a pharmaceutically acceptable derivative thereof, in admixture with a pharmaceutically acceptable adjuvant, diluent or carrier; and

(b) a pharmaceutical formulation including a prodrug of a low molecular weight thrombin inhibitor, or a pharmaceutically acceptable derivative of that prodrug, in admixture with a pharmaceutically acceptable adjuvant, diluent or carrier,

which components (a) and (b) are each provided in a form that is suitable for administration in conjunction with the other.

It is preferred that the prodrug of component (b) is a prodrug of the active low molecular weight thrombin inhibitor of component (a).

According to a further aspect of the invention, there is provided a method of making a kit of parts as defined herein, which method comprises bringing a component (a), as defined above, into association with a component (b), as defined above, thus rendering the two components suitable for administration in conjunction with each other.

By bringing the two components "into association with" each other, we include that components (a) and (b) may be:

(i) provided as separate formulations (i.e. independently of one another), which are subsequently brought together for use in conjunction with each other in combination therapy; or

(ii) packaged and presented together as separate components of a "combination pack" for use in conjunction with each other in combination therapy.

Thus, there is further provided a kit of parts comprising:

(1) one of components (a) and (b) as defined herein; together with

(2) instructions to use that component in conjunction with the other of the two components.

The kits of parts defined herein may comprise more than one formulation including an appropriate quantity/dose of thrombin inhibitor, and/or more than one formulation including an appropriate quantity/dose of respective prodrug, in order to provide for repeat dosing. If more than one formulation (comprising thrombin inhibitor or prodrug) is present, such

formulations may be the same, or may be different in terms of the dose of thrombin inhibitor/prodrug, chemical composition and/or physical form.

A further aspect of the invention provides a method of treatment of a condition in which inhibition of thrombin is required or desired, which comprises administration of:

(a) a pharmaceutical formulation including a low molecular weight thrombin inhibitor, or a pharmaceutically acceptable derivative thereof, in admixture with a pharmaceutically acceptable adjuvant, diluent or carrier; in conjunction with

(b) a pharmaceutical formulation including a prodrug of a low molecular weight thrombin inhibitor, or a pharmaceutically acceptable derivative of that prodrug, in admixture with a pharmaceutically acceptable adjuvant, diluent or carrier,

to a patient suffering from, or susceptible to, such a condition.

For the avoidance of doubt, as used herein, the term "treatment" includes therapeutic and/or prophylactic treatment.

"Pharmaceutically acceptable derivatives" of thrombin inhibitors and prodrugs includes salts (e.g. pharmaceutically acceptable non-toxic organic or inorganic acid addition salts) and solvates. It will be appreciated that the term pharmaceutically acceptable derivatives of active thrombin inhibitors includes those derivatives that have the same biological function and/or activity as that thrombin inhibitor but, for the purposes of this invention, does not include prodrugs of that thrombin inhibitor.

By "administration in conjunction with", we include that respective formulations comprising thrombin inhibitor and/or prodrug are administered, sequentially, separately and/or simultaneously, over the course of treatment of the relevant condition, which condition may be acute or chronic. Preferably, the term includes that the two formulations are administered (optionally repeatedly) sufficiently closely in time for there to be a beneficial effect for the patient, that is greater, over the course of the treatment of the relevant condition, than if either of the two formulations are administered (optionally repeatedly) alone, in the absence of the other formulation, over the same course of treatment. Determination of whether a combination provides a greater beneficial effect in respect of, and over the course of treatment of, a particular condition, will depend upon the condition to be treated or prevented, but may be achieved routinely by the skilled person.

Thus, the term "in conjunction with" includes that one or other of the two formulations may be administered (optionally repeatedly) prior to, after, and/or at the same time as, administration with the other component. When used in this context, the terms "administered simultaneously" and "administered at the same time as" include that individual doses of thrombin inhibitor and prodrug are administered within 48 hours (e.g. 24 hours) of each other.

Components (a) and (b) as described herein may also be presented (i.e. formulated) as a combined preparation (i.e. presented as a single formulation including low molecular thrombin inhibitor and prodrug).

Thus, there is further provided a pharmaceutical formulation including a low molecular weight thrombin inhibitor (or a pharmaceutically acceptable

derivative thereof) and a prodrug of a low molecular weight thrombin inhibitor (or a pharmaceutically acceptable derivative of that prodrug), in admixture with a pharmaceutically acceptable adjuvant, diluent or carrier.

The term "low molecular weight thrombin inhibitor" will be understood by those skilled in the art. The term may also be understood to include any composition of matter (e.g. chemical compound) which inhibits thrombin to an experimentally determinable degree in *in vivo* and/or in *in vitro* tests, and which possesses a molecular weight of below 2,000, preferably below 1,000.

Preferred low molecular weight thrombin inhibitors include low molecular weight peptide-based, amino acid-based, and/or peptide analogue-based, thrombin inhibitors.

The term "low molecular weight peptide-based, amino acid-based, and/or peptide analogue-based, thrombin inhibitors" will be well understood by one skilled in the art to include low molecular weight thrombin inhibitors with one to four peptide linkages, and includes those described in the review paper by Claesson in Blood Coagul. Fibrin. (1994) 5, 411, as well as those disclosed in US Patent N° 4,346,078; International Patent Applications WO 93/11152, WO 93/18060, WO 93/05069, WO 94/20467, WO 94/29336, WO 95/35309, WO 95/23609, WO 96/03374, WO 96/06832, WO 96/06849, WO 96/25426, WO 96/32110, WO 97/01338, WO 97/02284, WO 97/15190, WO 97/30708, WO 97/40024, WO 97/46577, WO 98/06740, WO 97/49404, WO 97/11693, WO 97/24135, WO 97/47299, WO 98/01422, WO 98/57932, WO 99/29664, WO 98/06741, WO 99/37668, WO 99/37611, WO 98/37075, WO 99/00371, WO 99/28297, WO 99/29670, WO 99/40072, WO 99/54313, WO 96/31504, WO

00/01704 and WO 00/08014; and European Patent Applications 648 780, 468 231, 559 046, 641 779, 185 390, 526 877, 542 525, 195 212, 362 002, 364 344, 530 167, 293 881, 686 642, 669 317, 601 459 and 623 596, the disclosures in all of which documents are hereby incorporated by reference.

Preferred low molecular weight peptide-based thrombin inhibitors include HOOC-CH_2-(*R*)Cha-Pic-Nag-H (wherein Cha represents cyclohexylalanine, Pic represents (*S*)-pipecolinic acid and Nag represents noragmatine; known as inogatran; see International Patent Application WO 93/11152) and, especially, HOOC-CH_2-(*R*)Cgl-Aze-Pab-H (known as melagatran; see above and International Patent Application WO 94/29336).

The term "prodrug" of a low molecular weight thrombin inhibitor includes any compound that, following oral or parenteral administration, is metabolised *in vivo* to form a low molecular weight thrombin inhibitor (as defined herein), in an experimentally-detectable amount, and within a predetermined time (e.g. within a dosing interval of between 6 and 24 hours (i.e. once to four times daily)), following oral or parenteral administration. Prodrugs of the thrombin inhibitor melagatran that may be mentioned include those disclosed in international patent application WO 97/23499. Preferred prodrugs are those of the formula R^1O_2C-CH_2-(*R*)Cgl-Aze-Pab-OH (see the list of abbreviations above or in WO 97/23499), wherein R^1 represents C_{1-10} alkyl or benzyl, such as linear or branched C_{1-6} alkyl (e.g. C_{1-4} alkyl, especially methyl, propyl and, particularly, ethyl) and the OH group replaces one of the amidino hydrogens in Pab.

The term "condition in which inhibition of thrombin is required or desired" will be understood by those skilled in the art to include the following:

The treatment and/or prophylaxis of thrombosis and hypercoagulability in blood and tissues of animals including man. It is known that hypercoagulability may lead to thrombo-embolic diseases. Conditions associated with hypercoagulability and thrombo-embolic diseases which may be mentioned include inherited or acquired activated protein C resistance, such as the factor V-mutation (factor V Leiden), and inherited or acquired deficiencies in antithrombin III, protein C, protein S, heparin cofactor II. Other conditions known to be associated with hypercoagulability and thrombo-embolic disease include circulating antiphospholipid antibodies (Lupus anticoagulant), homocysteinemi, heparin induced thrombocytopenia and defects in fibrinolysis.

The treatment of conditions where there is an undesirable excess of thrombin without signs of hypercoagulability, for example in neurodegenerative diseases such as Alzheimer's disease.

Particular disease states which may be mentioned include the therapeutic and/or prophylactic treatment of venous thrombosis (e.g. DVT) and pulmonary embolism, arterial thrombosis (e.g. in myocardial infarction, unstable angina, thrombosis-based stroke and peripheral arterial thrombosis) and systemic embolism usually from the atrium during arterial fibrillation or from the left ventricle after transmural myocardial infarction, or caused by congestive heart failure; prophylaxis of re-occlusion (ie thrombosis) after thrombolysis, percutaneous trans-luminal

angioplasty (PTA) and coronary bypass operations; the prevention of re-thrombosis after microsurgery and vascular surgery in general.

Further indications include the therapeutic and/or prophylactic treatment of disseminated intravascular coagulation caused by bacteria, multiple trauma, intoxication or any other mechanism; anticoagulant treatment when blood is in contact with foreign surfaces in the body such as vascular grafts, vascular stents, vascular catheters, mechanical and biological prosthetic valves or any other medical device; and anticoagulant treatment when blood is in contact with medical devices outside the body such as during cardiovascular surgery using a heart-lung machine or in haemodialysis; the therapeutic and/or prophylactic treatment of idiopathic and adult respiratory distress syndrome, pulmonary fibrosis following treatment with radiation or chemotherapy, septic shock, septicemia, inflammatory responses, which include, but are not limited to, edema, acute or chronic atherosclerosis such as coronary arterial disease, cerebral arterial disease, peripheral arterial disease, reperfusion damage, and restenosis after percutaneous trans-luminal angioplasty (PTA).

Preferred conditions include thrombosis, especially DVT, including distal and proximal DVT. The present invention finds particular utility in the prophylactic treatment of DVT resulting from surgery, such as gastrointestinal, or orthopaedic, surgery (e.g. hip or knee replacement). This includes DVT resulting from immobilisation after surgery.

In accordance with the invention, thrombin inhibitors, prodrugs of thrombin inhibitors, and derivatives of either, may be administered orally, intravenously, subcutaneously, buccally, rectally, dermally, nasally, tracheally, bronchially, topically, by any other parenteral route, or *via*

inhalation, in the form of a pharmaceutical preparation comprising the thrombin inhibitor or prodrug in a pharmaceutically acceptable dosage form. Depending on the disorder, and the patient, to be treated, as well as the route of administration, the compositions may be administered at varying doses.

Preferred modes of delivery are systemic. For melagatran and derivatives thereof, preferred modes of administration are parenteral, more preferably intravenous, and especially subcutaneous. For prodrugs of melagatran, preferred modes of administration are oral.

In the therapeutic treatment of mammals, and especially humans, thrombin inhibitors, prodrugs of thrombin inhibitors, and derivatives of either will generally be administered as a pharmaceutical formulation in admixture with a pharmaceutically acceptable adjuvant, diluent or carrier, which may be selected with due regard to the intended route of administration and standard pharmaceutical practice.

Suitable formulations for use in administering thrombin inhibitors are known in the art, and include those known from US Patent N° 4,346,078; International Patent Applications WO 93/11152, WO 93/18060, WO 93/05069, WO 94/20467, WO 94/29336, WO 95/35309, WO 95/23609, WO 96/03374, WO 96/06832, WO 96/06849, WO 96/25426, WO 96/32110, WO 97/01338, WO 97/02284, WO 97/15190, WO 97/30708, WO 97/40024, WO 97/46577, WO 98/06740, WO 97/49404, WO 97/11693, WO 97/24135, WO 97/47299, WO 98/01422, WO 98/57932, WO 99/29664, WO 98/06741, WO 99/37668, WO 99/37611, WO 98/37075, WO 99/00371, WO 99/28297, WO 99/29670, WO 99/40072, WO 99/54313, WO 96/31504, WO 00/01704 and WO 00/08014; and

European Patent Applications 648 780, 468 231, 559 046, 641 779, 185 390, 526 877, 542 525, 195 212, 362 002, 364 344, 530 167, 293 881, 686 642, 669 317, 601 459 and 623 596, the disclosures in all of which documents are hereby incorporated by reference.

Suitable formulations for use with melagatran, derivatives and prodrugs thereof are described in the literature, for example as described in *inter alia* international patent applications WO 94/29336, WO 96/14084, WO 96/16671, WO 97/23499, WO 97/39770, WO 97/45138, WO 98/16252, WO 99/27912 and WO 99/27913, the disclosures in which documents are hereby incorporated by reference. Otherwise, the preparation of suitable formulations may be achieved non-inventively by the skilled person using routine techniques.

The amounts of thrombin inhibitor, prodrug, or derivative of either, in the formulation will depend on the severity of the condition, and on the patient, to be treated, as well as the compound(s) which is/are employed, but may be determined non-inventively by the skilled person.

Suitable doses of thrombin inhibitors, prodrugs and derivatives of either, in the therapeutic and/or prophylactic treatment of mammalian, especially human, patients may be determined routinely by the medical practitioner or other skilled person, and include the respective doses discussed in the prior art documents disclosing thrombin inhibitors that are mentioned hereinbefore, the disclosures in which are hereby incorporated by reference.

In the case of melagatran, suitable doses of active compound, prodrugs and derivatives thereof, in the therapeutic and/or prophylactic treatment of

mammalian, especially human, patients include those which give a mean plasma concentration of up to 5 μmol/L, for example in the range 0.001 to 5 μmol/L over the course of treatment of the relevant condition. Suitable doses may thus be in the range 0.1 mg once daily to 25 mg three times daily, and/or up to 100 mg infused parenterally over a 24 hour period, for melagatran, and in the range 0.1 mg once daily to 100 mg three times daily for prodrugs of melagatran including those specifically mentioned hereinbefore.

In any event, the physician, or the skilled person, will be able to determine the actual dosage which will be most suitable for an individual patient, which is likely to vary with the condition that is to be treated, as well as the age, weight, sex and response of the particular patient to be treated. The above-mentioned dosages are exemplary of the average case; there can, of course, be individual instances where higher or lower dosage ranges are merited, and such are within the scope of this invention.

The sequence in which the formulations comprising thrombin inhibitor, and prodrug, may be administered (i.e. whether, and at what point, sequential, separate and/or simultaneous administration takes place) may be determined by the physician or skilled person. For example, the sequence may depend upon many factors that will be evident to the skilled person, such as whether, at any time during the course or period of treatment, one or other of the formulations cannot be administered to the patient for practical reasons (e.g. the patient is unconscious and thus unable to take an oral formulation comprising either thrombin inhibitor or prodrug).

For example, in the treatment of thrombosis (e.g. DVT) resulting from surgery, such as gastrointestinal, or orthopaedic, surgery, and when the active thrombin inhibitor is melagatran, it is preferred that the formulation comprising melagatran is administered parenterally within two days (e.g. within 24 hours) of surgery (either prior to or after surgery), and particularly immediately prior to (e.g. within 2 hours), and/or within up to 12 hours after, surgery (e.g. at least one hour after surgery), and thereafter for up to between 3 and 7 (e.g. between 0 and 2, such as between 1 and 2) days after that surgery, and that the formulation comprising prodrug is administered orally within 7 days following that surgery (preferably once administration of melagatran has been terminated) for up to e.g. between 11 and 40 days, preferably 9 days, more preferably up to 8 days.

The method described herein may have the advantage that, in the treatment of conditions in which inhibition of thrombin is required or desired, it may be more convenient for the physician and/or patient than, be more efficacious than, be less toxic than, have a broader range of activity than, be more potent than, produce fewer side effects than, or that it may have other useful pharmacological properties over, similar methods known in the prior art for the treatment of such conditions.

The invention is illustrated, but in no way limited, by the following example.

Example 1

Clinical Trial - Melagatran and EtOOC-CH_2-(*R*)Cgl-Aze-Pab-OH Combination Therapy

A controlled, randomised, parallel group, Swedish multi-centre pilot study was carried out. The study was open with regard to the drugs under evaluation but was blind for the patients, all personnel at the study sites, and for the person monitoring the experiments with regard to the doses of melagatran and the prodrug of melagatran, EtOOC-CH_2-(*R*)Cgl-Aze-Pab-OH (P; see WO 97/23499).

Dalteparin (Fragmin®; Pharmacia-Upjohn) was used as a reference compound.

Patients scheduled for primary elective total hip or knee replacement were eligible for inclusion, and were randomly selected into one of three groups, each to receive different doses of melagatran and P, or dalteparin. In all, 135 patients were included in the study, of which 105 patients could be used for evaluation with respect to thromboembolic events using central assessment of locally performed phlebograms.

About 32 patients in each treatment group were evaluated according to the protocol. A stratified randomisation, by centre and type of surgery, was used to ensure that approximately equal numbers of patients were given each of the drugs under evaluation at all participating centres (in all six centres were used) for both types of surgery (hip or knee). Each centre received study drugs in blocks of four, separately for hips and knees. Within each block, the order of the study drugs was randomised.

The following formulations were used in the study:

Melagatran – 5, 10 or 20 mg/mL in aqueous saline solution.

P - appropriate weight (see below) in a tablet also comprising 59 to 63 mg corn starch, 115 mg microcrystalline cellulose and 2 mg sodium stearyl fumarate.

The following doses of melagatran and P were used in the study:

Treatment A - s.c. melagatran (1 mg) b.i.d. for 2 days, followed by oral administration of P (6 mg) b.i.d. for 6 to 9 days.

Treatment B - s.c. melagatran (2 mg) b.i.d. for 2 days, followed by an oral administration of P (12 mg) b.i.d. for 6 to 9 days.

Treatment C - s.c. melagatran (4 mg) b.i.d. for 2 days, followed by an oral administration of P (24 mg) b.i.d. for 6 to 9 days.

The patients receiving melagatran and P received treatment on the day of surgery. The patient received the first injection after induction of anaesthesia immediately before surgery. For knee-patients, the pre-operative melagatran injection was given before tourniquets were applied. The second injection was given in the evening the same day. The patient received one melagatran injection in the morning and one in the evening over the next 24 hours, until oral administration of P, twice daily, started. The first oral dose of P was always taken in the morning. Thus, the total treatment period was between 8 and 11 days.

Treatment D - dalteparin (Fragmin®): one s.c. injection of 5000 U during the evening of the day before surgery, continuing with one s.c. injection every evening over a treatment period of 8 to 11 days.

The plasma concentrations of melagatran were recorded.

The results of the trial, in terms of the frequencies of thromboembolism after hip or knee surgery, are tabulated below:

	Treatment A		Treatment B		Treatment C		Treatment D	
	(n)	(%)	(n)	(%)	(n)	(%)	(n)	(%)
Outcome	6/29	21	6/24	25	4/24	16	5/27	19

These data show that a combination of subcutaneously administered melagatran and orally administered P is effective in preventing DVT after orthopaedic surgery.

Claims

1. A kit of parts comprising:

(a) a pharmaceutical formulation including a low molecular weight thrombin inhibitor, or a pharmaceutically acceptable derivative thereof, in admixture with a pharmaceutically acceptable adjuvant, diluent or carrier; and

(b) a pharmaceutical formulation including a prodrug of a low molecular weight thrombin inhibitor, or a pharmaceutically acceptable derivative of that prodrug, in admixture with a pharmaceutically acceptable adjuvant, diluent or carrier,

which components (a) and (b) are each provided in a form that is suitable for administration in conjunction with the other.

2. A kit of parts as claimed in Claim 1, wherein the prodrug of component (b) is a prodrug of the thrombin inhibitor of component (a).

3. A kit of parts as claimed in Claim 1 or Claim 2, wherein components (a) and (b) are suitable for sequential, separate and/or simultaneous use in the treatment of a condition in which inhibition of thrombin is required or desired.

4. A kit of parts as claimed in Claim 3, wherein the condition is deep venous thrombosis.

5. A kit of parts as claimed in any one of Claims 1 to 4, wherein the thrombin inhibitor is melagatran.

6. A kit of parts as claimed in Claim 5, wherein the prodrug is of the formula

$$R^1O_2C\text{-}CH_2\text{-}(R)Cgl\text{-}Aze\text{-}Pab\text{-}OH,$$

wherein R^1 represents linear or branched C_{1-6} alkyl and the OH group replaces one of the amidino hydrogens in Pab.

7. A kit of parts as claimed in Claim 6, wherein R^1 represents methyl, ethyl or propyl.

8. A kit of parts as claimed in any one of the preceding claims, wherein the formulation comprising thrombin inhibitor, or derivative thereof, is a parenteral formulation and that comprising the prodrug, or derivative thereof, is an oral formulation.

9. A method of making a kit of parts as defined in any one of Claims 1 to 8, which method comprises bringing a component (a) according to any one of Claims 1 to 8, into association with a component (b) according to any one of Claims 1 to 8, thus rendering the two components suitable for administration in conjunction with each other.

10. A kit of parts comprising:

(1) one of components (a) and (b) as defined in any one of Claims 1 to 8; together with

(2) instructions to use that component in conjunction with the other of the two components.

11. A pharmaceutical formulation including a low molecular weight thrombin inhibitor (or a pharmaceutically acceptable derivative thereof) and a prodrug of a low molecular weight thrombin inhibitor (or a

pharmaceutically acceptable derivative of that prodrug), in admixture with a pharmaceutically acceptable adjuvant, diluent or carrier.

12. A method of treatment of a condition in which inhibition of thrombin is required or desired, which comprises administration of:

(a) a pharmaceutical formulation including a low molecular weight thrombin inhibitor, or a pharmaceutically acceptable derivative thereof, in admixture with a pharmaceutically acceptable adjuvant, diluent or carrier; in conjunction with

(b) a pharmaceutical formulation including a prodrug of a low molecular weight thrombin inhibitor, or a pharmaceutically acceptable derivative of that prodrug, in admixture with a pharmaceutically acceptable adjuvant, diluent or carrier,

to a patient suffering from, or susceptible to, such a condition.

13. A method as claimed in Claim 12 in which component (a) is administered prior to commencement of administration of component (b).

14. A method of treatment of a condition in which inhibition of thrombin is required or desired, which comprises administration of a formulation as defined in Claim 11 to a patient suffering from, or susceptible to, such a condition.

15. A method as claimed in any one of Claims 12 to 14, wherein the condition is deep venous thrombosis.

16. A method as claimed in Claim 15, wherein the thrombosis results from surgery.

17. A method as claimed in Claim 16, wherein the surgery is gastrointestinal surgery or orthopaedic surgery.

18. A method as claimed in Claim 16 or Claim 17, wherein component (a) is administered parenterally prior to and/or after surgery and component (b) is administered orally following that surgery.

19. The use of a thrombin inhibitor, or a pharmaceutically acceptable derivative thereof, in the manufacture of a medicament for the treatment or prophylaxis of a condition in which inhibition of thrombin is required or desired, which treatment or prophylaxis comprises administration of:

(a) a pharmaceutical formulation including a low molecular weight thrombin inhibitor, or a pharmaceutically acceptable derivative thereof, in admixture with a pharmaceutically acceptable adjuvant, diluent or carrier; in conjunction with

(b) a pharmaceutical formulation including a prodrug of a low molecular weight thrombin inhibitor, or a pharmaceutically acceptable derivative of that prodrug, in admixture with a pharmaceutically acceptable adjuvant, diluent or carrier,

to a patient suffering from, or susceptible to, such a condition.

Atty Dkt

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RULE 63 (37 C.F.R. 1.63)
DECLARATION AND POWER OF ATTORNEY
FOR PATENT APPLICATION
IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

As a below named inventor, I hereby declare that my residence, post office address and citizenship are as stated below next to my name, and I believe I am the original first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled: NEW USE

the specification of which (**check** applicable **box(s)**):

☐ is attached hereto

☐ was filed on _____ as U.S. Application Serial No. _____ (Atty Dkt. No.)

☒ was filed as PCT international appl. No. SE00/00756 on 19 April 2000

a n d (if applicable to U.S. or PCT application) was amended on _____

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above. I acknowledge the duty to disclose information which is material to the patentability of this application in accordance with 37 C.F.R. 1.56. I hereby claim foreign priority benefits under 35 U.S.C. 119/365 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed or, if no priority is claimed, before the filing date of this application:

Prior Foreign Application(s):

Application Number	**Country**	**Day/Month/Year Filed**
9901442-5	Sweden	21 April 1999
9904419-0	Sweden	3 December 1999

I hereby claim the benefit under 35 U.S.C. §119(e) of any United States provisional application(s) listed below.

Application Number	**Day/Month/Year Filed**

I hereby claim the benefit under 35 U.S.C. 120/365 of all prior United States and PCT international applications listed above or below and, insofar as the subject matter of each of the claims of this application is not disclosed in such prior applications in the manner provided by the first paragraph of 35 U.S.C. 112, I acknowledge the duty to disclose material information as defined in 37 C.F.R. 1.56 which occurred between the filing date of the prior applications and the national or PCT international filing date of this application:

Prior U.S./PCT Application(s): Application Serial No.	**Day/Month/Year Filed**	**Status: patented, pending, abandoned**

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon. And I hereby appoint **NIXON & VANDERHYE P.C., 1100 North Glebe Rd., 8th Floor, Arlington, VA 22201-4714, telephone number (703) 816-4000 (to whom all communications are to be directed)**, and the following attorneys thereof (of the same address) individually and collectively my attorneys to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith and with the resulting patent: Arthur R. Crawford, 25327; Larry S. Nixon, 25640; Robert A. Vanderhye, 27076; James T. Hosmer, 30184; Robert W. Faris, 31352, Richard G. Besha, 22770; Mark E. Nusbaum, 32348; Michael J. Keenan, 32106; Bryan H. Davidson, 30251; Stanley C. Spooner, 27393; Leonard C. Mitchard, 29009; Duane M. Byers, 33363; Paul J. Henon, 33626; Jeffry H. Nelson, 30481; John R. Lastova, 33149; H. Warren Burnam, Jr., 29366; Thomas E. Byrne, 32205; Mary J. Wilson, 32955; J. Scott Davidson, 33489; Jerry D. Craig, 38026; Alan M. Kagen, 36178; William J. Griffin, 31260.

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